Interactive Effects of Metals and PAHs on Benthic Food Webs

Kevin R. Carman (PI)
Dept. of Biological Sciences
Louisiana State University
Baton Rouge, LA 70803
phone: 225-578-1761 fax: 225-578-2597 email: zocarm@lsu.edu

Co-PIs
John W. Fleeger
Dept. of Biological Sciences
Louisiana State University
Baton Rouge, LA 70803
phone: 225-578-1240 fax: 225-578-2597 email: zoflee@lsu.edu

Robert P. Gambrell
Dept. of Oceanography and Coastal Sciences
Louisiana State University
Baton Rouge, LA 70803
phone: 225-578-6426 fax: 225-578-6307 email: cowgam@unix1.sncc.lsu.edu

Ralph J. Portier
Institute for Environmental Studies
Louisiana State University
Baton Rouge, LA 70803
phone: 225-578-4287 fax: 225-578-4286 email: rportie@lsu.edu

Award Number: N00149910023
http://www.biology.lsu.edu/faculty_listings/fac_pages/kcarman.html

LONG-TERM GOALS

Our long-term goals are to understand how complex mixtures of contaminants influence benthic communities at the levels of microorganisms, microalgae, invertebrate grazers, and fish predators. In particular, we are interested in how contaminants influence foodweb interactions among these groups of organisms.

OBJECTIVES

Our research examines the interactive effects of metal (Cu, Cr, Cd, Hg, and Pb) and diesel-fuel contaminants on the benthic food web of a coastal salt marsh and the specific role that Cu plays in this suite of contaminants. Specifically, we are examining how diesel and metal contaminants interact to influence the microbial (bacteria and microalgae), invertebrate, and juvenile fish components of the benthic community, and how their interactions influence trophic relationships among organisms. Previous studies have focused on either the ecotoxicological effects of metals *or* the effects of hydrocarbons, but essentially nothing is known about how these two classes of contaminants interact. The modes of toxicity of hydrocarbons and metals are quite different, and individually they may elicit different, sometimes opposite, ecological responses. Impacted field sediments, especially in harbors,

Report Documentation Page			*Form Approved OMB No. 0704-0188*
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1. REPORT DATE **30 SEP 2001**	2. REPORT TYPE		3. DATES COVERED **00-00-2001 to 00-00-2001**
4. TITLE AND SUBTITLE **Interactive Effects of Metals and PAHs on Benthic Food Webs**			5a. CONTRACT NUMBER
			5b. GRANT NUMBER
			5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)			5d. PROJECT NUMBER
			5e. TASK NUMBER
			5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Dept. of Biological Sciences,,Louisiana State University,,Baton Rouge,,LA,70803**			8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION/AVAILABILITY STATEMENT **Approved for public release; distribution unlimited**			
13. SUPPLEMENTARY NOTES			
14. ABSTRACT			
15. SUBJECT TERMS			

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**7**	

are typically contaminated with both metals and hydrocarbons, and thus ecological impacts may be a consequence of their interactive effects. Our experimental approach to this problem will provide fundamental information on the ecological manifestations of metals-hydrocarbons interactions, and provide the basis for making ecologically sound decisions concerning appropriate bioremediation or mitigation strategies for contaminated field sites.

APPROACH

Experimental work consists primarily of microcosm experiments to examine contaminant effects on benthic microbes and grazers. Additional laboratory experiments are performed to determine toxicological effects of contaminants on individual organisms. As proposed, two major microcosms experiments were performed in year 1 to examine responses of, and interactions between microbes and meiofauna when exposed to metals and diesel-metal combinations under normoxic conditions. In year 2, a microcosm experiment was performed to determine the specific role of Cu in mixtures of contaminants, and the influence of fish bioturbators (*Gobiosoma bosci*) on the influence of contaminants. In the past year we have performed species toxicity experiments, and focused on analysis of data and preparation of manuscripts.

The microcosm approach used is one that we developed to study the influence of contaminants on interactions between microbes and meiofauna of sediment food webs (e.g., Carman et al. 1997). The microcosms (15.2 cm i.d.) represent minimally disturbed, natural assemblages of benthic organisms and the sediment in which they live. Experiments are well replicated (n = 4-5 per treatment) and include a complete set of uncontaminated controls, which allows rigorous hypothesis testing.

Microcosms of marsh sediment were collected by hand from mudflats associated with a *Spartina alterniflora* saltmarsh near the LUMCON facility in Cocodrie, LA and treated with diesel and metal contaminants as generally described by Carman et al. (1997). In the experiment conducted in May/June of 2000, experimental treatments were as follows. Sediments were contaminated with known concentrations of Navy-relevant metals - Cu, Cr, Cd, Pb, and Hg and or diesel-contaminated sediments. Metal concentrations were manipulated to simulate the relative abundances of metals in San Diego Harbor (SDH) (219 ppm Cu, 178 ppm Cr, 1 ppm Cd, 51 ppm Pb, 1 ppm Hg; Kennish 1997). Each treatment was represented by 4 replicate microcosms, and exposures were 30 days. The treatments were:

Treatment	Description
Controls	No contaminants
Diesel	Diesel only
High Metals	Cu, Cr, Cd, Hg, & Pb; 6x [SDH]
Low Metals	Cu, Cr, Cd, Hg, & Pb; 2x [SDH]
High Cu	Cu; 6x [SDH]
High Metals-Cu	Cr, Cd, Hg, & Pb; 6x [SDH]
High Metals + Diesel	Diesel and Cu, Cr, Cd, Hg, & Pb; 6x [SDH]
Low Metals + Diesel	Diesel and Cu, Cr, Cd, Hg, & Pb; 2x [SDH]
High Cu + Diesel	Diesel and Cu; 6x [SDH]
Low Cu + Diesel	Diesel and Cu; 2x [SDH]
High Metals-Cu + Diesel	Diesel and Cr, Cd, Hg, & Pb; 6x [SDH]
Low Metals-Cu + Diesel	Diesel and Cr, Cd, Hg, & Pb; 2x [SDH]

Natural photoperiods and temperature were maintained. Samples were collected to determine: (1) bacterial biomass and community composition (collaboration with D.C. White, Univ. of Tennessee), (2) microalgal abundance, community composition, and productivity, (3) meiofaunal abundance and

community composition, (4) meiofaunal grazing on microalgae, (5) sediment hydrocarbon concentrations (6) bulk sediment and acetate-extractable concentrations, (7) pore-water Cu and Cu speciation, (8) sediment carbohydrate and EPS concentration. In addition, we monitored O_2, and NH_4^+ concentrations in water overlying microcosms, and determined vertical profiles of oxygen and RedOx in sediment.

WORK COMPLETED

All experimental work has been completed. We are now in the process of analyzing data and preparing manuscripts. Seven oral presentations of our work were presented in 2001 at the following professional meetings: ASLO, Benthic Ecology, and International Association of Meiobenthologists. Other aspects of the work will also be presented at the SETAC and Estuarine Research Federation meetings during the fall of 2001.

RESULTS

At the highest concentrations, metals and diesel individually strongly reduced the abundance of meiofaunal taxa, and there was no evidence of non-additive effects in joint exposures. Exposure to a lower level of metals did not influence meiofaunal abundance at the level of major taxon (nematodes, ostracods, total copepods, nauplii, or chironomid larvae) or individual copepod species. Lower diesel exposures decreased abundances of ostracods, nauplii, and chironomids, and the combination of lower levels of metals and diesel yielded results similar to the effects of diesel alone. Lower diesel exposures did not significantly influence abundances of nematodes or total copepods. However, copepod species displayed a range of responses that were categorized into two groups: 'diesel-sensitive' species declined in abundance (presumably due to direct toxic effects), and 'diesel-resistant' species, whose abundances were either unchanged, or increased dramatically (due to indirect effects). Total-copepod abundance was significantly reduced in metals-diesel mixtures, suggesting a metals-diesel synergism. However, while the addition of metals eliminated the enhanced abundances of 'diesel-sensitive' species, responses of individual copepod species to metals-diesel combinations did not generally suggest a metals-diesel toxicological synergism. The presence of diesel enhanced the retention of metals in sediments, and influenced the speciation of Cu, which may have important toxicological implications. **Our observations indicate that, at relatively low (non-catastrophic) co-contaminant concentrations, both direct (toxic) and indirect effects (e.g., reduced competition, enhanced food availability) influence faunal abundance, and the analysis of major taxa may yield spurious conclusions concerning environmental impacts; lack of changes in total abundance may mask important changes in community composition, and reductions in abundance may give the appearance of toxicological synergisms that are not manifested at the species level.**

Our results also indicate that Cu plays a critical role in the ecotoxicological effects of metal, and metal-diesel mixtures. Of particular importance, we note that the effects of Cu, alone and in interaction with other metals and diesel, varies considerably among individual copepod species. Fig. 1 illustrates the response of one copepod species, *Pseudostenhelia wellsi*. At higher concentrations of a mixture of metals (Cu, Cd, Hg, Cr, Pb; red bar) abundances are decreased. Abundances are similarly reduced when exposed to Cu only (blue bar), implying that Cu is a primary toxicant in the mixture. When Cu is removed from the mixture of metals (light green bar), no adverse effects are observed. At a lower concentration of metals (pink bar), no toxic effects are observed, nor is diesel-contaminated sediment toxic (dark green bar; N.B. a relatively low level of diesel contamination was used here). However, when the mixture of low metals, or the lower concentration of Cu alone is combined with diesel (pink bar with cross hatch, (light blue bar with cross hatch, respectively), abundances are reduced.

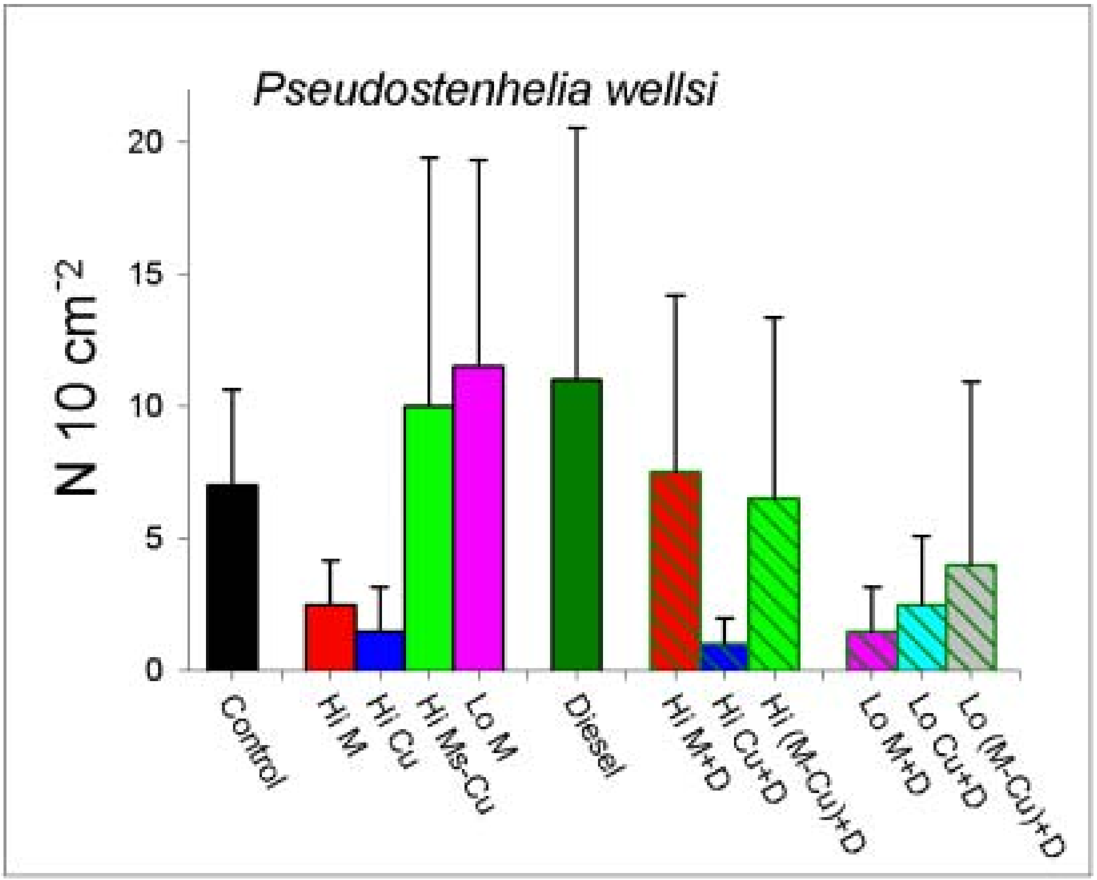

Fig. 1. Influence of contaminants on a benthic harpacticoid copepod within natural sediment microcosms.

We note briefly that each of copepod species examined displayed a unique response to the various contaminants and contaminant mixtures shown in Fig. 1, e.g., (a) *Microarthridion* sp. was more sensitive to metals/diesel mixtures when Cu was absent from metals mixtures (i.e., Cu had an antagonistic effect), (b) The abundance of *Cletocamptus* sp. increased dramatically in the presence of diesel, but this effect was dramatically reduced when metals were combined with diesel, (c) The abundance of *Halicyclops coulli* was either unaffected or enhanced in the presence of all contaminant treatments, (d) All metal/diesel mixtures had an adverse effect on *Paronychocamptus huntsmani*.

IMPACT/APPLICATIONS

Our results indicate that metals and hydrocarbons interact to produce unique toxicological effects on benthic microalgae and invertebrates. Especially at lower contaminant concentrations, the effects of individual and mixed contaminants can be subtle and highly species specific, resulting in major changes in community composition. Indirect (i.e., non-toxicological) effects appear to be particularly important in reshaping communities; specifically, the abundances of many species *increase* in the presence of low levels of contaminants, apparently a consequences of reduced competition from more sensitive species, or enhanced availability of food from algal blooms. Cu is of particular importance to metal-hydrocarbon interactions, but again, its effects are highly variable and species specific. Some

copepod species suffer high mortality when exposed to low concentrations of Cu, while the abundance of others increase. Some are sensitive to Cu when it is combined with low levels of diesel. For at least one species, Cu appears to play an antagonistic role, i.e., metal-diesel combinations are toxic only when Cu is *absent*. It is also clear that Cu speciation in sediment porewaters strongly influences its toxicological effect. In Millward et al. (2001), we showed that toxic effects on benthos are strongly related to the availability of inorganic Cu (Cu') in porewaters. Our results also show that diesel reduces the strength with which ligands bind to dissolved Cu, thus rendering Cu more bioavailable and more toxic in metal-diesel mixtures.

TRANSITIONS

Roger Nisbet (UCSB) is heading a working group funded by the National Center for Ecological Analysis and Synthesis to develop models of contaminant effects on benthic food webs.
Results of our experimental work are being used in this on-going collaboration. We are also collaborating with D.C. White (Univ. Tenn.) to examine effects of contaminants on microbial community structure.

RELATED PROJECTS

1 - "How does produced water cause a reduction in the genetic diversity of harpacticoid copepods?" Minerals Management Service (J. Fleeger, PI and D. Foltz, Co-PI), $241,000, 1998-01. (An investigation into the influence of PAH's on genetic diversity of copepods.)

2 - "Relationships between benthic microalgae, grazers, and nutrients in a coastal salt marsh". NSF (K.R. Carman, PI.), $330,000. 1999-02. (Basic foodweb relationships in the system being studied in the ONR project).

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